Australian Journal of Crop Science AJCS
AJCS 7(6):849-854 (2013) ISSN:1835-2707

Cytomorphological and molecular evidences of synthesis of interspecific hybrids between *Brassica rapa* and *B. fruticulosa* through sexual hybridization

Arun Kumar, Binay K. Singh*, Vijay V. Singh and Jitendra S. Chauhan

Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur -321 303, Rajasthan (India)

***Corresponding author: binaybio@gmail.com**

Abstract

Six successful interspecific hybrid plants were obtained through sexual hybridization between *Brassica rapa* var. yellow sarson (NRCYS-05-02) (2n = 20, AA) and a wild species *B. fruticulosa* (2n = 16, FF), using the latter as pollen parent. Morphological, cytological and sequence tagged microsatellite sites (STMS) based molecular analyses were carried out to confirm the hybrid nature of F_{1s} plants. The hybrid plants (2n = 18) were intermediate for most of the morphological attributes. A severe reduction (8.6%) in pollen fertility was recorded in F_1. Nevertheless, few seeds were obtained from open pollination. Meiotic analysis revealed a mixture of bivalents and univalents in all the pollen mother cells (PMCs) analysed. However, 2II + 14I was the most frequently observed chromosome association. Presence of large number of univalents indicates lack of homology between pairing partners. Besides, bivalents and univalents, a trivalent was also observed in three PMCs, indicating segmental homology between chromosomes. The study suggests that *B. fruticulosa* has partial genome homoeology with *B. rapa* which could be exploited in crop improvement programmes, particularly breeding for tolerance to insect pests, especially mustard aphid.

Keywords: Homoeologuos pairing; meiotic analysis; molecular markers; pollen fertility; wide hybridization.
Abbreviations: LSD - Least significant difference; PMCs - Pollen mother cells; STMS - Sequence tagged microsatellite sites.

Introduction

Brassica is economically one of the most important genus of the family *Brassicaceae* (Cardoza and Stewart, 2004). It constitutes important sources of vegetables, cooking oil and condiments (Lim et al., 2006). Narrow genetic variability in crop Brassicas, caused mainly due to intensive selection over past several decades, has jeopardized the crop improvement programmes (Cowling, 2007; Ananga et al., 2008). Fortunately, *Brassica* coenospecies has been bestowed with nearly 100 species and genera of wild and weedy relatives, serving as rich reservoir of genes conferring many agriculturally important traits. These species can be effectively utilized to introduce economically important traits to cultivated species as well as development of potential wide hybrids (Prakash, 2001; Bang et al., 2007). *Brassica rapa* L., which is one of the important oil crops, holds great economic importance throughout the world. It contributes 10 A genome chromosomes to amphidiploid oilseed species such as *Brassica napus* L. (2n = 38, AACC) and *Brassica juncea* (L.) Czern & Coss. (2n = 36, AABB) (U, 1935). Three ecotypes of *B. rapa* (2n = 20, AA) i.e. yellow sarson, brown saron and toria are widely grown as oilseed crops in India (Prakash and Chopra, 1996). These are well adapted to dry and arid ecosystems and reported to mature earlier than other Brassicas (Kimber and McGregor, 1995). It is also reported that they are vulnerable to severe yield penalty due to mustard aphid (*Lipaphis erysimi*) and lack of potential plant genotypes which are suitable for intensive cultivation (Prakash and Raut, 1983). Notably, *Brassica fruticulosa* Cyr. subsp. *fruticulosa* (2n = 16, FF), a wild relative of cultivated Brassicas, show significant resistance against mustard aphid (Ellis et al., 2000; Kumar et al., 2011). Present study was aimed at developing interspecific hybrids of *B. rapa* and *B. fruticulosa*, having useful genetic attributes of putative parents, to generate genetic variability and introgression of genes with special reference to resistance against mustard aphid. This investigation reports successful synthesis of six interspecific hybrids between *B. rapa* var. yellow sarson (NRCYS-05-02) and *B. fruticulosa*, and evidences for its successful establishment through morphological, cytological and molecular marker assisted analyses. The genomic homology and differentiation pattern based upon crossability, degree of meiotic chromosome pairing, fertility factors and STMS analysis is also being reported. STMS markers, due to their co-dominant nature, have been extensively used as a robust and reliable system for confirming hybridity in distant hybridization programmes. To the best of our knowledge, this is the first report of interspecific hybrids between *B. rapa* var. yellow sarson (NRCYS-05-02) and *B. fruticulosa* through sexual hybridization, using *B. fruticulosa* as a male parent.

Results

Hybridization, and morphological characterization of parents and F_1 hybrids

The F_1 hybrids between *B. rapa* and *B. fruticulosa* were obtained when *B. rapa* was used as female parent. One hundred and forty eight buds were pollinated from which 11 siliquae with a total of 20 seeds were obtained. The hybrid seeds were small in size, similar to those of *B. fruticulosa*, and light brown in colour. Seeds of the parents as well as F_1 hybrids were sown in pots maintained in field condition during the normal cropping season. Out of the 20 seeds only six seeds germinated and the plants survived till maturity, and thus the percentage success of crossability was 30%.

Table 1. Comparison of morphological characters of parents and their F_1 hybrids (*B. rapa* × *B. fruticulosa*).

Characters	*B. rapa*	F_1 hybrid	*B. fruticulosa*	LSD (5%)
Plant height (cm)	72.5	52.7*	57.7	4.82
Days to 50% flowering	72.0	68.0*	60.0	2.59
Number of primary branches per plant	6.6	5.7**	4.8	5.29
Number of secondary branches per plant	6.5	6.3**	8.6	1.17
Main raceme length (cm)	33.7	27.9*	32.8	1.93
Siliqua length (cm)	4.5	1.5*	2.9	0.74
Corolla length (cm)	0.7	0.5**	1.0	0.73
Corolla width (cm)	0.5	0.4**	0.5	0.71
Siliqua texture	Smooth	Smooth	Constricted (around the seeds)	-
Seed colour	Yellow	Light brown	Brown	-
Leaf colour	Medium green	Light green	Dark green	-
Leaf hairiness	Absent	Dense	Sparse	-
Leaf margin	Entire	Crenate	Crenate	-
Leaf lobes	Present	Present	Present	-
Type of petiole	Sessile	Petiolate	Petiolate	-
Petal colour	Yellow	Light yellow	Yellow	-

*Significant at p = 0.05, **non significant at p = 0.05.

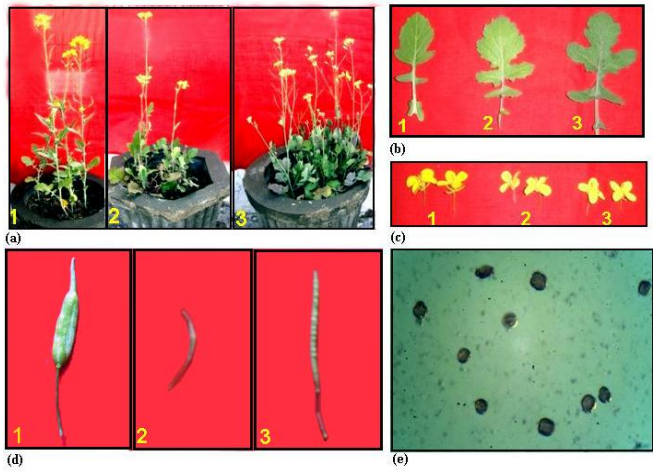

Fig 1. Comparison of morphological attributes of parents and their F_1 hybrids. (1a) Parents and hybrid plants, 1 - *B. rapa*, 2 - F_1 hybrids, 3 - *B. fruticulosa*. (1b) Comparative leaf morphology, 1 - *B. rapa*, 2 - F_1 hybrids, 3 - *B. fruticulosa*. (1c) Comparative flower morphology, 1 - *B. rapa*, 2 - F_1 hybrids, 3 - *B. fruticulosa*. (1d) 1 - *B. rapa*, 2 - F_1 hybrids, 3 - *B. fruticulosa*. (1e) Pollen stainablity of F_1 hybrids.

The F_1 plants were medium in height, profusely branched and intermediate to their parents for most of the morphological and inflorescence attributes (Table 1, Fig 1). The leaves were light green in colour with dense hair, petiolate, lobed and lyrately pinnatified. The dentation of leaf margin was noted to be crenate type with obtuse tip and petal colour (light yellow) was intermediate to the parents. The hybrid plants had smaller siliquae as compared to the parents. The LSD test at 5% level of significance for plant height, days to 50% flowering, siliqua length and main raceme length of the hybrid plants, as compared to the parents, were recorded to be significant. However, it was noted to be non significant for the number of primary and secondary branches per plant, and flower size. Few seeds were set in the F_1 plants. However, no seed set was achieved under self-pollination or in backcrosses. The F_1 plants harboured lower aphid populations than the highly susceptible female parent *B. rapa* var. yellow sarson (NRCYS-05-02).

Cytology of parents and their F_1 hybrids

Meiotic data of parents and F_1 hybrids has been summarized in Table 2 and Table 3, and illustrated in Fig 2. Both the species included in the interspecific hybridization were diploid in nature. Although the majority of pollen mother cells (PMCs) analyzed at diakinesis/metaphase I in parents showed normal bivalent formation while 30.6% cells had a mixture of univalents and bivalents. However, the number of univalents never exceeded six per cell. The average number of chromosome associations of *B. rapa* and *B. fruticulosa* was 9.29II + 1.33I and 7.41II + 0.91I, respectively. The disjunction of bivalents/chromosomes at anaphase I and II, by and large, was normal. Meiotic analysis of the F_1 hybrids of *B. rapa* and *B. fruticulosa* showed a mixture of univalents, bivalents and trivalents in a total of 54 PMCs analysed. Four PMCs (7.4%) had 18 univalents while remaining cells had bivalents and multivalent (trivalent) associations, in addition to the compensating number of univalents. The occurrence of univalents was most frequent. The average chromosome association in the hybrid was 0.06III + 3.34II + 14.19I. The number of univalents and bivalents ranged from 2 - 18 and 0 - 8, respectively, whereas number of trivalents never exceeded one per PMC. Numerous disjunctional abnormalities including late disjunction of bivalents, bridges and laggards were observed at anaphase I and II (Table 3). Sixty percent of the total PMCs analysed showed laggards at anaphase I. Bridge - fragment configuration was observed in two (6.67%) PMCs.

Table 2. Chromosome association at diakinesis/metaphase I and percentage pollen stainablity in PMCs of parents and their F_1 hybrids.

Species/hybrid	2n	PMCs observed	Chromosome associations									Percentage pollen stainablity
			Univalents			Bivalents			Trivalent			
		No.	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	
B. rapa	20	24	32	1.33	0-6	223	9.29	7-10	-	-	-	91.12
B. fruticulosa	16	22	20	0.91	0-4	163	7.41	6-8	-	-	-	83.23
B. rapa × *B. fruticulosa*	18	54	766	14.19	2-18	181	3.34	0-8	3	0.06	0-1	8.60

Table 3. Chromosome distribution at anaphase I and II (A I, II) in *B. rapa* × *B. fruticulosa* hybrids (2n = 18).

Chromosome distribution	PMCs observed	
	Number	Percentage
Equal distribution (9:9)	4	13.33
Laggards at A I	18	60.00
Laggards at A II	6	20.00
Bridge-fragment configuration	2	6.67
Total	30	

Table 4. List of STMS primers used for the analysis of F_1 hybrids.

Locus code	Repeat	Primer sequence (5′→3')	Locus code	Repeat	Primer sequence (5′→3')
Ra2-A01	$(GA)_{19}$	F - ttcaaaggataagggcatcg R - tcttcttcttttgttgtcttccg	BRMS-037	-	F - ctgctcgcatttttttatcatac R - tacgcttgggagagaaaactat
Ra2-E04	$(GA)_{19}$	F - acacacaacaaacagctcgc R - aacatcaaacctctcgacgg	BRMS-042-2	-	F - agctcccgacagcaacaaaaga R - ttcgcttccttttctgggaatg
Ra2-E11	$(CT)_{24}$	F - ggagccaggagagaagaagg R - cccaaaacttccaagaaaagc	BRMS-050	-	F - aactttgcttccactgattttt R - ttgcttaacgctaaatccatat

However, a few cells were recorded with normal distribution of chromosomes resulting in some fertile pollen grains in the hybrids.

Pollen stainability

The diploid parental species showed pollen stainability as high as 91.12% in *B. rapa* and 83.23% in *B. fruticulosa*. However, the F_1 hybrids showed drastic reduction in pollen stainability, recording only 8.60% (Table 2). The reductions in pollen stainability in hybrid plants indicate a high degree of pollen sterility, possibly due to meiotic irregularities and segregational anomalies.

Confirming hybridity using STMS markers

STMS marker analysis of genomic DNA was carried out to establish the hybrid nature of the F_1 plants. These markers have emerged as the marker of choice for molecular genetic applications due to their abundant and uniform distribution throughout the genome, highly variable nature with regard to repeat number, co-dominant inheritance, ease of transferability and reproducibility, and a very high efficiency in revealing the hybrid nature of F_1s. In the present study, six genomic STMS markers were used amongst which Ra2-A01 was found to be polymorphic between the parents. The amplicons specific to both the parents were present in the F_1 plants in a co-dominant manner, establishing the hybridity of the F_1s (Fig 3).

Discussion

Interspecific hybridization between cultivated species of *Brassica* and their wild relatives offers a potential opportunity for introgression of desired traits from wild to the cultivated forms (Prakash and Chopra, 1988). In the present study, six interspecific hybrid plants were obtained through sexual hybridization between *B. rapa* var. yellow sarson (NRCYS-05-02) and wild species *B. fruticulosa*. Interspecific hybrid plants were obtained when *B. fruticulosa* was used as a male parent, and all the hybrids grew up to maturity. The perusal of literature available indicates the production of interspecific hybrids in *Brassica* mostly through embryo rescue techniques (Chandra et al., 2004; Harberd and McArthur, 1980). Moreover, interspecific/ intergeneric hybrids are successfully produced only when wild species are used as female (Chandra et al., 2004; Chen et al., 2011). Contrary to this, the present investigation reports the successful hybridization between *B. rapa* and *B. fruticulosa* even when wild species (*B. fruticulosa*) is involved as a male parent. It is quite useful in maintaining the cytological background of the crop species in distant hybridization programmes. The F_1 hybrid plants thus obtained were found to be intermediate as compared to parent species with respect to many morphological and inflorescence attributes. These observations are in congruence with earlier published reports (Chen et al., 2011; Choudhary and Joshi, 2001). The occurrence of characteristics from both progenitor species in the hybrids indicates that the F_1 plants inherited genomes of both parental species crossed. In the present investigation, six STMS markers derived from *B. rapa*, and observed to be cross-transferable to *B. fruticulosa*, were used to establish the hybrid nature of the synthesized plants. However, only one STMS marker was able to distinguish the parents and showed its presence in co-dominant manner in the hybrids.

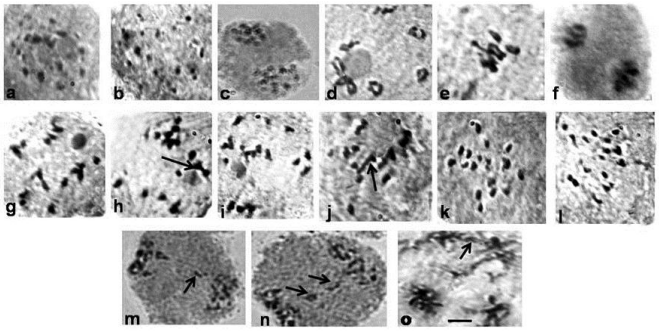

Fig 2. Meiotic analysis of parents and their F_1 hybrids showing chromosome associations at diakinesis/metaphase I, and chromosome distribution at anaphase I and II. (a-c) *B. rapa*, (a-b) Diakinesis 10II, (c) Anaphase I 10:10 (equal distribution). (d-f) *B. fruticulosa*, (d) Diakinesis 8II, (e) Metaphase I 8II, (f) Anaphase I 8:8 (equal distribution). (g-o) F_1 hybrids, (g) Diakinesis 3II+12I, (h) Diakinesis 1III+15I (trivalent marked by arrow), (i) Diakinesis 4II+11I, (j) Diakinesis 1III+1II+13I (trivalent marked by arrow), (k) Metaphase I 1II+16I, (l) Metaphase I 18I, (m-n) Anaphase I with laggards (marked by arrow), (o) Anaphase II with bridge (marked by arrow). Bar: 10 µm.

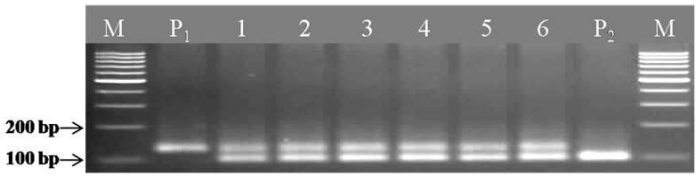

Fig 3. STMS analysis of parents and their F_1 hybrids with the primer Ra2-A01. Lane M - 100 bp DNA ladder, Lane P_1- *B. rapa* var. yellow sarson (NRCYS-02-05), Lanes 1-6 - F_1 hybrids, Lane P_2 - *B. fruticulosa.* The co-appearance of DNA bands present in parents P_1 and P_2 in samples 1-6 indicate their hybrid nature.

At five out of the six loci which were analysed, both the parental species showed monomorphism, depicting a similarity of about 83% at the genomic level. These two species have already been reported to share monomorphism (72%) on the basis of chloroplast and mitochondrial DNA profile (Pradhan et al., 1992). The cytological analysis of the parents revealed a sporadic occurrence of univalents in diploid species of *B. rapa* and *B. fruticulosa*. This may be a direct consequence of precocious separation of rod bivalents, as reported in a number of plants (Sybenga, 1972). Cytological analysis of F_1 hybrids (2n = 18) not only confirmed their hybridity but also indicated extent of genome homoeology between the parents. From the meiotic analysis of hybrids, it was observed that PMCs had chromosome association on average of 0.06III + 3.34II + 14.91I. A maximum of four bivalents per PMC was recorded, but 2II + 14I was the most common meiotic configuration. The F_1 hybrids showed predominance of univalents, which is typical of wide hybrids. The observation of bivalents which ranged between 0-8 in various PMCs in the hybrids could be interpreted to be due to archaic homology within the chromosomes of the same genome (autosyndesis). However, the occurrence of higher associations in the form of trivalents, though only in few PMCs, can be attributed to allosyndesis indicating partial homoeology or segmental

allopolyploidy between the two parental genomes (Prakash et al., 2009). Thus, it appears *B. fruticulosa* (FF) has some homoeology with *B. rapa* (AA), indicating possibility of gene inrogression from *B. fruticulosa* to *B. rapa* through conventional means. Occasional laggards were recorded at anaphase I and the bridge fragment configuration at anaphase II in two PMCs. It is similar to the study reported by Choudhary and Joshi (2001). The possibility of occurrence of such phenomenon results from chiasma formation within a heterozygous inversion. Such chiasmata demonstrate true homology and pairing between the genomes involved (Attia and Robbelen, 1986). Drastic decrease in pollen fertility and reduced seed set recorded in the present hybrids might be due to meiotic irregularities and segregational anomalies (Singh, 1993). The occurrence of homoeologous pairing between *B. rapa* and *B. fruticulosa* chromosomes and few seeds set by interspecific hybrids offers an opportunity for the transfer of useful genes across the species.

Materials and methods

Plant material

Seed samples of *B. rapa* var. yellow sarson (NRCYS-05-02) and *B. fruticulosa* were obtained from the germplasm section of the Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur, Rajasthan, India.

Hybridization

Field grown plants of the cultivated *B. rapa* var. yellow sarson (NRCYS-05-02) and *B. fruticulosa* were used to produce interspecific hybrids. Unopened flower buds of *B. rapa* were emasculated in the afternoon, covered with paper bags and pollinated with freshly collected pollens of *B. fruticulosa* in the following morning and rebagged. The seeds collected from the crosses as well as from the parents were sown in pots under field conditions. Morphological comparisons were made and hybridity of the F_1s was ascertained by cytological as well as STMS marker analyses. Morphological data was analyzed by LSD test at 5% level of significance.

Cytological analysis

For meiotic observations flower buds of an appropriate size were collected from mature plant and fixed in freshly prepared carnoy's fluid (ethanol : chloroform : acetic acid - 6 : 3 : 1), supplemented with a drop of ferric chloride solution, for a minimum of 24 hours at room temperature and subsequently stored in 70% alcohol at 10°C. For meiotic analysis anthers were squashed in 1% acetocarmine and a total of 22, 24 and 54 PMCs obtained from parents and hybrids, respectively, were analyzed at diakinesis/metaphase I stages. For percentage pollen stainability, the pollen grains were stained in 1:1 (glycerine: acetocarmine) mixture and on an average five slides were scored for stainable pollen grains. Normal pollen grains were round, densely stained and were distinguishable from small, shrunken and lightly stained sterile pollen grains.

Molecular analysis

Primer sets for six STMS markers derived from *B. rapa*, which are cross-transferable to *B. fruticulosa* (Singh et al., 2012), were custom synthesized and used to establish the hybridity of the synthesized plants. The details of the primers including their origin, repeat motif and nucleotide sequence are indicated in Table 4. The PCR amplification reactions were performed using 50 ng of genomic DNA, extracted by the procedure described by Murray and Thompson (1980), in a total volume of 25 µl containing 0.2 µM of each primer, 0.2 mM of each dNTP, 1.0-2.5 mM $MgCl_2$, and 1.5 U Taq polymerase. Touchdown PCR protocol was used with 30 cycles at 94°C for 30 s, 65-56°C for 30 s, and 72°C for 5 s. The annealing temperature started at 65°C and dropped by 0.3°C each cycle, followed by three cycles with annealing at 56°C. The PCR products were visualized in 3.5%, MetaPhor (FMC BioProducts, Rockland, ME, USA) agarose gels containing 0.5 ng/ml of ethidium bromide.

Conclusion

B. rapa is one of the most important oilseed crops distributed worldwide. This crop suffers heavy yield losses due to damage caused by a wide range of insect pests and diseases. Most notable among them are mustard aphid, and white rust and *Alternaria* blight caused by *Albugo candida* and *Alternaria brassicae*, respectively. In this sense, distant hybridization with *B. fruticulosa*, a wild relative of cultivated Brassicas, is a useful approach to transfer useful traits across the species. However, a very limited cross-compatibility exists between these two species. Even with the embryo rescue techniques the number of hybrids obtained is rather limited. Present study reports successful interspecific hybridization between *B. rapa* var. yellow sarson (NRCYS-05-02) and *B. fruticulosa*, using the latter as pollen donor. The hybrid nature of the F_1 plants was also established through morphological, cytological and STMS based molecular analyses. Currently, these hybrids are being multiplied by *in vitro* methods, as very few seeds were collected from the F_1 hybrids. These hybrid lines would be valuable genetic resources not only to breed more productive cultivars resistant to the above mentioned biotic factors, but also to analyze each chromosome and gene concerned.

Acknowledgments

We sincerely acknowledge Director, Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur - 321 303 (Rajasthan) India, for providing financial support and the facilities to carry out this research work.

References

Ananga A, Cebert E, Soliman K, Kantety R, Konan K, Oshieng JW (2008) Phylogenetic relationships within and among *Brassica* species from RAPD loci associated with blackleg resistance. Afr J Biotechnol. 7:1287-1293

Attia T, Robbelen G (1986) Cytogenetic relationship with cultivated *Brassica* analyzed in amphihaploids from the three diploid ancestors. Can J Genet Cytol. 28:223-329

Bang SW, Sugihara K, Jeung BH, Kaneko R, Satake E, Kaneko Y, Matsuzawa Y (2007) Production and characterization of intergeneric hybrids between *Brassica oleracea* and a wild relative *Moricandia arvensis*. Plant Breeding. 126:101-103

Cardoza V, Neal CS Jr (2004) *Brassica* Biotechnology: Progress in cellular and molecular biology. *In vitro* Cell Dev - Pl. 40:542-551

Chandra A, Gupta ML, Banga SS, Banga SK (2004) Production of an interspecific hybrid between *Brassica fruticulosa* and *B. rapa*. Plant Breeding. 123:497-498

Chen JP, Ge XH, Yao XC, Feng Ye-H, Li ZY (2011) Synthesis and characterization of Interspecific trigenomic hybrids and allohexaploids between three cultivated *Brassica* allotetraploids and wild species *Brassica fruticulosa*. Afr J Biotechnol. 10:12171-12176

Choudhary BR, Joshi P (2001) Crossability of *Brassica tournefortii* and *B. rapa*, and morphology and cytology of their F_1 hybrids. Theor Appl Genet. 102:1123-1128

Cowling WA (2007) Genetic diversity in Australian canola and implications for crop breeding for changing future environments. Field Crop Res. 104:103-111

Ellis PR, Kiff NB, Pink DAC, Jukes PL, Lynn J, Tatchell GM (2000) Variation in resistance to the cabbage aphid (*Brevicoryne brassicae*) between and within wild and cultivated *Brassica* species. Genet Resour Crop Ev. 47:395-401

Harberd DJ, McArthur ED (1980) Meiotic analysis of some species and genus hybrids in the *Brassiceae*. In: Tsunoda S, Hinata K, Gómez-Campo C (ed) *Brassica* crops and wild allies. Japan Scientific Societies Press, Tokyo

Kimber DS, McGregor DI (1995) The species and their origin, cultivation and world production. In: Kimber DS, McGregor DI (ed) *Brassica* oilseeds, production and utilization. CAB Int, Wallingford

Kumar S, Atri C, Sangha MK, Banga SS (2011) Screening of wild crucifers for resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) and attempt at introgression of resistance gene(s) from *Brassica fruticulosa* to *Brassica juncea*. Euphytica. 179:461-470

Lim YP, Plaha P, Choi SR, Uhm T, Hong CP, Bang JW, Hur YK (2006) Toward unraveling the structure of *Brassica rapa* genome. Physiol Plantarum. 126:585-591

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4326

Pradhan AK, Prakash S, Mukhopadhyay A, Pental D (1992) Phylogeny of *Brassica* and allied genera based on variation in chloroplast and mitochondrial DNA patterns: molecular and taxonomic classifications are incongruous. Theor Appl Genet. 85:331-340

Prakash S (2001) Utilization of wild germplasm of *Brassica* allies in developing cytoplasmic male sterility- fertility restoration systems in Indian mustard *Brassica juncea*. In: Liu H, Fu TD (ed): Proceedings of International Symposium Rapeseed Science. Science Press, New York

Prakash S, Bhat SR, Qurios CF, Kirit PB, Chopra VL (2009) *Brassica* and its close allies: cytogenetics and evolution. Plant Breed Rev. 31:21-187

Prakash S, Chopra VL (1988) Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through nonhomologous recombination. Plant Breeding. 101:167-168

Prakash S, Chopra VL (1996) Origin and evaluation. In: Chopra VL, Prakash S (ed) Oilseed and vegetable *Brassicas*: Indian perspective. Oxford & IBH Pub, New Delhi

Prakash S, Raut RN (1983) Artificial synthesis of *Brassica napus* and its prospects as an oilseed crop in India. Ind J Genet. 43:282-290

Singh BK, Thakur AK, Rai PK (2012) Genetic diversity and relationships in wild species of *Brassica* and allied genera as revealed by cross-transferable genomic STMS marker assays. Aus J Crop Sci. 6:815-821

Singh RJ (1993) Plant Cytogenetics. CRC Press Inc. Bocca Raton, Florida, USA

Sybenga J (1972) General cytogenetics. North Holland Publishing Company, Amsterdam

UN (1935) Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot. 7:389-452